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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/090,320	03/01/2002	Yanxiang Cao	3446	5376

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EXAMINER	
ZHOU, SHUBO	
ART UNIT	**PAPER NUMBER**
1631	

DATE MAILED: 02/27/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/090,320	CAO ET AL.	
	Examiner	Art Unit	
	Shubo (Joe) Zhou	1631	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 November 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-6 and 10-29* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6 and 10-29* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Applicants' amendments and request for reconsideration in the communication filed on 11/30/05 are acknowledged and the amendments entered.

Claims 1-6 and 10-29 are currently pending and under consideration.

2. The rejection of claims 1-6 and 10-29 under 35 U.S.C. 112 , second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention set forth in the previous Office action mailed 6/30/05 is hereby withdrawn in view of applicants' amendment to claim 1.

Claim Rejections-35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later

invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

4. Claims 1-4, 6, and 10-29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lockhart et al. (US Patent No. 6,040,138, Date of Patent: Mar 21, 2000, filing date: Sep. 15, 1995) in view of Pharmacia Biotech (Molecular and Cell Biology Product Catalog, 1994) and Williams et al. (Nucleic Acids Research, Vol. 22, pages 1365-1367, 1994), further in view of Stahl et al. (The Journal of Histochemistry and Cytology, Vol. 41, pages 1735-1740, 1993).

The claims are drawn to a method of analyzing an RNA sample comprising converting the RNA into cDNAs with random primers and reverse transcriptase, which cDNAs are then hybridized to nucleic acid probes which can identify two different isoforms from a target gene a sample. The method comprises fragmenting the cDNAs for labeling.

Lockhart et al. teach a method of monitoring gene expression by hybridization of cDNAs derived from total RNA or mRNAs of biological samples by reverse transcription using oligo dT primers to high density oligonucleotide arrays. See columns 4, 11, 12. However, Lockhart et al. do not explicitly teach that random primers are used for the reverse transcription and the cDNA synthesized for hybridization to the probes on the array are fragmented. Lockhart et al. also do not explicitly disclose providing isoform specific probes for mRNA isoform detection in a sample.

Pharmacia provides commercial kits for synthesizing cDNA from RNA for various purposes. Pharmacia provides TimeSaver cDNA Synthesis Kit comprising both Oligo dT primers and random hexamers. The instruction teaches that random primers are useful for making cDNAs that increase the representation of 5' end of an RNA, or for copying mRNAs lacking a poly(A) tail.

Williams et al. teach that dangling ends of a duplex formed by the hybridization of the two oligonucleotides have unpredictable effect on the stability of the duplex, depending on the

location and composition of the dangling ends. See Abstract, page 1365, Figure 1 on page 1366, and the Discussion on page 1367.

Stahl et al. provide a method for selection of oligonucleotide probes for detection of mRNA isoforms. See page 1735, Abstract and page 1736, left column. Stahl et al. states that using oligonucleotides for the detection of isoforms have clear advantages over cloned fragments such as low costs. See page 1735, right column.

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the method of Lockhart et al. to use random primers in lieu of, or in addition to the oligo dT primers to take advantage of using random primers in reverse transcription so that the cDNA produced have a better representation of the 5' end of an RNA molecule as suggested by Pharmacia. One having ordinary skill in the art would also have been motivated by Williams et al. to modify the method of Lockhart et al. to fragment the cDNAs before labeling to generate labeled cDNA fragments that are similar to the lengths of the probes on the oligonucleotide array in order to minimize the dangling ends of the duplex formed after hybridization so that a better consistency can be achieved as to the signal intensities obtained from a sample and/or among multiple samples.

As to comprising probes on the array to detect RNA isoforms of a gene, while Lockhart et al. do not explicitly including isoform specific probes on the array, they do disclose that a multiplicity of probes are provided on a high density array where each probe is complementary to a subsequence of the target nucleic acid. The multiplicity probes can include every different probe of length that is complementary to a subsequence of the target nucleic acid. The probes can range from about 10 to about 50 nucleotides in length. See column 5. It would have been obvious to one having ordinary skill in the art that the array would have been useful for isoform detection because with an array comprising such a multiplicity of probes with short sequences that are complementary to unique subsequences of a target gene, some of the multiplicity probes

will hybridize to one isoform but not others because the probes are short (10-50 nucleotides long) and are complementary to only short subsequences.

One of ordinary skill in the art would have been motivated by Stahl et al. to provide isoform-specific oligonucleotides on the array disclosed by Lockhart et al. in order to study the expression of different mRNA isoforms of a gene.

As to claim 2, which requires that the number of cDNA copies of a given sequence near the 3' end of an RNA is not more than twice the number of cDNA copies of a given sequence near the 5'end of the RNA molecule, it would have been obvious to a person having ordinary skill in the art at the time the invention was made that since the random primers used for priming the RNA into cDNA would be relatively uniformly distributed to an RNA molecule during reverse transcription, and as suggested by Pharmacia that the use of random primers increases the representation of the 5' end of an RNA molecule, the number of cDNA copies of a given sequence near the 3' end of the RNA would not be more than twice the number of cDNA copies of a given sequence near the 5'end of the RNA molecule, hence the hybridization signal detected with a probe to a 3' region of an RNA would not be more than twice the amount of signal detected with a probe to a 5' region of the RNA.

As to claims 3, 10, 15-20, which require the RNA sample comprises a particular type of RNA or from a particular source, Lockhart et al. teach that the RNA sample can be total RNA, or mRNA or poly(A)+ RNA. See columns 2-3, 10 and 11. Further, Lockhart et al. teach that the RNA sample can be from any organism, any biological tissues or cells, or clinical samples, or sections of tissues or frozen sections. See columns 11-12.

As to claim 14, which requires that the RNA sample is isolated from a prokaryotic cell, a person having ordinary skill in the art would have been motivated to use the method of Lockhart et al. and use random primer for the synthesis of cDNA from RNA of a prokaryotic source because Lockhart et al teach that their method can be used for RNA samples from any source

(see above), and Pharmacia teaches that reverse transcription with random primer would be useful for copying mRNA lacking a poly(A) tail, which is the case for prokaryotic RNA.

As to claims 11-13, which require that the random primers used for reverse transcriptions are 6, 9, or 15 nucleotides in length, it would have been obvious to one of ordinary skill in the art that the exact length of the random primer can vary in the cDNA synthesis because different length of random primers have been used in the prior art. For example, the kits of Gibco BRL and Pharmacia comprise random hexamers (6mer); Malfroy-Camine et al. (US 5,780,025, date of patent: Jul. 14, 1998) teach using random octamers in the synthesis of cDNA from RNA (see column 17); and Lader et al. (US 6,057,134) disclose using random decamers for reverse transcription to synthesize cDNA (see column 6). Thus, one of ordinary skill in the art would be motivated to try various lengths of random primers such as, 6mers, 9mers or 15mers to see whether better synthesis would be achieved.

This rejection is reiterated from the previous Office action mailed 6/30/05. Applicants' argument filed 11/30/05 has been fully considered but is not found persuasive. The argument is on the ground that Williams et al. would not motivate fragmenting cDNAs prior to hybridization. Applicants argue that "in no case did they observe a destabilizing effect of an overhang" and that "this stabilizing effect of the 2 base overhang compared to the 1 base overhang was seen with only with a GA overhang at the 5' end of the target-GA was the ony two base 5' overhang tested. A 3' overhang of CA showed no effect on hybridization." See pages 7-8 of the response. This is not found persuasive because as set forth in the previous Office action and above, Williams et al. show that dangling ends of a duplex formed by the hybridization of the two oligonucleotides have unpredictable effect on the stability of the duplex, depending on the location and composition of the dangling ends. The motivation by Williams et al. to modify Lockhart et al. is not to generate overhangs to increase or decrease hybridization intensity but rather to minimize

overhangs to increase consistency because as admitted by applicants, Williams show that the effect of overhangs on hybridized duplex stability is unpredictable depending on which end of the duplex the overhang locates. Thus, one having ordinary skill in the art would have been motivated to fragment the cDNA prior to hybridization to increase hybridization consistency. Applicants further argue that assuming arguendo that Williams et al. suggest that eliminating overhang is advantageous, fragmenting with DNase I would not result in the defined fragment sized required to achieve consistency. This is also not found persuasive because fragmenting with DNase I is not a limitation in the claims. Rather, the claims such as claim 1 simply recite generic method of fragmenting. It would have been obvious to one having ordinary skill in the art that there would be many well-known methods of fragmenting nucleic acids to generate fragments with size same or smaller than those of the probes on the microarray of Lockhart et al. such as mechanical fragmenting or even with DNase I. The sizes of the fragments could be monitored by gel electrophoresis, etc.

5. Claim 5 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lockhart et al. (US Patent No. 6,040,138, Date of Patent: Mar 21, 2000, filing date: Sep. 15, 1995) in view of Pharmacia Biotech (Molecular and Cell Biology Product Catalog, 1994), Williams et al. (Nucleic Acids Research, Vol. 22, pages 1365-1367, 1994) and Stahl et al. (The Journal of Histochemistry and Cytology, Vol. 41, pages 1735-1740, 1993) as applied to claims 1-4, 6, and 10-29 above, further in view of Gibco BRL (Terminal Deoxynucleotidyl Transferase, Gibco BRL Catalog and Reference Guide, 1992).

The claim is drawn to a method of analyzing an RNA sample comprising converting the RNA into cDNAs with random primers and reverse transcriptase, which cDNAs are then fragmented and labeled by the addition of at least one labeled nucleotide using terminal transferase before being hybridized to nucleic acid probes on a solid support.

Applied to claims 1-4, 6, and 10-29 above, Lockhart et al., Pharmacia Biotech teach or suggest a method of monitoring gene expression by hybridization of cDNAs derived from total RNA or mRNAs of biological samples by reverse transcription using random primers to high density oligonucleotide arrays. However, the references do not explicitly teach that the cDNA fragments are labeled by the addition of at least one labeled nucleotide using terminal transferase.

Lockhart et al. teach that the labels of the cDNAs can be made with any of the means known to those of skill in the art such as end labeling.

Gibco BRL discloses and provides a terminal deoxynucleotidyl transferase. The instruction for the product states that the enzyme is "suitable for adding momopolymer tails to the 3' end of DNA" or "for labeling the 3' ends". See page 290.

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the method of Lockhart et al. to use terminal transferase to end label the cDNA fragments because Lockhart et al. clearly motivates and suggests end labeling and Gibco BRL provides the terminal transferase enzyme for exactly this purpose.

This rejection is reiterated from the previous Office action mailed 6/30/05. Applicants' argument filed 11/30/05 has been fully considered but is not found persuasive. The argument for this rejection is the same as that provided for the rejection at paragraph 4. The argument is not deemed persuasive for the same reasons as set forth above.

Conclusion

6. No claim is allowed.

7. **THIS ACTION IS MADE FINAL.**

8. Applicants are reminded of the extension of time policy as set forth in 37 C.F.R. §1.136 (a). A shortened statutory period for response to this final action is set to expire three months from the date of this action. In the event a first response is filed within two months of the mailing date of this final action and the advisory action is not mailed until after the end of the three-month shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 C.F.R. §1.136 (a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than six months from the mailing date of this final action.

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Shubo (Joe) Zhou, whose telephone number is 571-272-0724. The examiner can normally be reached Monday-Friday from 8 A.M. to 4 P.M. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ardin Marschel, Ph.D., can be reached on 571-272-0718. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to Patent Analyst Tina Plunkett whose phone number is (571) 272-0549.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the

problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public. For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

Shubo (Joe) Zhou, Ph.D.

Patent Examiner

18 February 2006

JOHN S. BRUSCA, PH.D
PRIMARY EXAMINER